Fission track age of zircon separates of tuffaceous mudstones of the Upper Siwalik subgroup of Jammu–Chandigarh sector of the Panjab Sub-Himalaya

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Fission track ages of zircon grains separated from tuffaceous mudstone/bentonitic tuffs of the Upper Siwalik subgroup of Chandigarh (Ghaggar section) and Jammu (Uttarbeni) are determined to be 2.14±0.51 Ma and 1.59±0.32 Ma respectively. The zircon grains are considered to be cogenetic with the ash falls, and hence provide age estimates of the eruptive event(s). Implications of these age estimates in the understanding of chronologic framework of the Upper Siwalik subgroup of the Jammu–Chandigarh sector of the Himalayan Foreland are briefly discussed.

TUFFACEOUS mudstones were reported from the Pinjor Formation of the Upper Siwalik subgroup in the area east of Chandigarh[1]. On the basis of the stratigraphic occurrence of the tuffaceous mudstones towards the base of the Pinjor Formation in the Ghaggar section, it was suggested[1] that these may correspond to the ash couplet dated between 2.4 ±0.20 Ma and 2.58 ±0.06 Ma in the Siwalik Group of Pakistan[2]. This surmise has now been investigated by fission track (FT) dating of zircon separates of the mudstones. FT ages of bentonitized tuffs from Nagrota Formation of Utterbeni area in Jammu Hills though reported in the literature are without any experimental and analytical details[3,4]. In view of this the FT age of zircon grains from bentonitic tuff bed from Utterbeni has also been attempted. Zircon was chosen for FT dating as it is fairly resistant to the process of thermal annealing of tracks. Present estimates place the long-term (10^6–10^7 years) annealing temperature of zircon at 225°–240° C (ref. 5). This value is sufficiently high for thermal events of lesser duration. The zircon grains are considered to be cogenetic with the ash fall(s) and should, therefore, date the eruptive event(s) and contemporaneous sedimentation. Further, burial of these tuffs to much less than 1 km for times of the order 10^6–10^7 years should have had no detectable fading effect on the spontaneous track densities of the zircon grains. They should, therefore, record a primary age.

Bulk sample (1–2 kg) was crushed and dispersed in water. Following wet sieving to remove clay minerals, the mineral concentrate was further sized to separate 60–200 mesh fraction. The zircon grains were separated using the conventional magnetic and heavy-liquid separation techniques.

FT dating was carried out at the Kurukshetra University Fission Track Dating Laboratory using external detector method in which the spontaneous fission tracks are etched and counted in the polished inner surface of the crystal and the induced fission tracks on an external muscovite detector (almost free from uranium) attached to the crystal during thermal neutron irradiation[6,7].

The euhedral and clear grains of zircon (considered to be cogenetic with a volcanic event) were mounted in PFA teflon. The mineral mount(s) were ground on a wet emery stone and polished with diamond pastes of 8 μm through 0.25 μm to obtain smooth surfaces with 4π geometry. The mounts were etched in an eutectic KOH–NaOH melt at 230°C for 26 h. In order to ensure optimum etching, the mounts were progressively etched till the appearance of fresh tracks stopped. The mineral mounts as well as two mounts of the standard dosimeter-glass CN1 (Corning-1) were covered with muscovite detectors and packed in an aluminium capsule. The capsule was irradiated in the IC2 thermal column of the CIRUS Reactor at Bhabha Atomic Research Centre, Bombay with a dose of $\sim 10^{15}$ n/cm^2. After irradiation, the muscovite detectors were etched in 48% HF at 30° C for 6 min to reveal the induced tracks.

The counting of tracks was done under Nikon optiphot microscope using 100× dry objective. The grain surfaces of high etching efficiency recognized from the existence of sharp polishing scratches[8], were used for counting.

FT age was calculated using the zeta calibration approach. Experimental measurements of the zeta factor in the FT laboratory of Kurukshetra University for the dosimeter glass CN1 using the internationally accepted age standards and IC2 thermal column of CIRUS reactor has been determined to be equal to 110.56±1.28 (1σ) (ref. 9). FT age was calculated using the following equation:

$$T = \frac{1}{\lambda_D} \ln \left[1 + \lambda_D \frac{G \zeta \rho_s \rho_d}{\rho_i} \right],$$

where λ_D is the total decay constant of uranium = 1.55125×10^{-10} yr^{-1} (ref. 10); ζ the zeta factor; ρ_s the spontaneous track density; ρ_i, ρ_d the induced track density in muscovite detectors attached to zircon sample and glass-dosimeter respectively; and G the geometry factor.

In order to estimate the errors, the data were subjected to χ^2-test[11] to detect the extra Poissonian error in track counts. The data failed the χ^2-test and hence instead of applying conventional analyses, the mean of the individual crystal track density ratios

Table 1. Fission track analytical data

Sample and locality	No of crystals and mineral	Lab. code	Track counts: Spontaneous		Induced		Glass dosimeter		$P(\chi)2$	Mean ρ_s/ρ_i	Coefficient of correlation (r)	Age ± 1σ (Ma)	Weighted mean Age ± 1σ (Ma)
			ρ_s	N_s	ρ_i	N_i	ρ_d	N_d					
Tuffaceous mudstone (Ghaggar section)	18 Zircon	V1ZR1	0.1902	35	10.25	1886	2.088	2610	<1%	0.0185	0.967	2.14 ± 0.51	2.14 ± 0.51
Bentonitized tuffs (Nagrota Formation of Utterbeni area) in Jammu Hills	12 Zircon	V4ZR1	0.22	66	8.16	2447	1.09	1644	<5%	0.027	0.980	1.63 ± 0.48	1.59 ± 0.32
	14 Zircon	V4ZR2	0.29	86	10.69	3121	1.03	1287	<5%	0.028	0.980	1.57 ± 0.43	

Track densities ρ are measured and are (10^6 tr cm^{-2}), N is the number of tracks counted.
Analysis done by external detector method with geometry factor $G = 0.5$
Age calculated using CN1 glass dosimeter and $\zeta_{CN1} = 110.56 \pm 1.28$ (1σ)
$P(\chi^2)$ is probability of obtaining χ^2 value for ν degrees of freedom (where ν = no. of crystals − 1).

(ρ_s/ρ_i) and the error on the mean ratio were calculated[12]. The error associated with the ratio is larger than that obtained by conventional analysis. The error in the age was then calculated by using the following equation:

$$\sigma_T = T\left[\left\{\frac{\sigma\overline{(\rho_s/\rho_i)}}{(\rho_s/\rho_i)}\right\}^2 + \left(\frac{\sigma_\zeta}{\zeta}\right)^2 + \frac{1}{N_d}\right]^{\frac{1}{2}},$$

where N_d is the total number of tracks counted in the muscovite detector attached to the glass dosimeter.

In the case of Jammu sample, the crop of zircon grains was sufficient enough to enable its multiple dating. The same age of two mounts dated under different irradiations indicates the appropriateness of the experimental procedure. The countring data and FT ages are given in Table 1.

The age of 2.14 ± 0.51 Ma is based on an average obtained from the analysis of eighteen zircon grains (which did not show much variation in track density ratio ρ_s/ρ_i) from the tuffaceous mudstone of the Ghaggar section. In terms of the large error envelop, the sample may range in age from 1.6 Ma to 2.6 Ma. This range in age allows the possibility of equating the tuff couplet of the Ghaggar section with that of Pakistan and Jammu related to the Gauss-Matuyama boundary.

Altered tuff horizons occur in the Siwalik Group in Pakistan between 3.0 Ma and 1.5 Ma (ref. 2). These occur in the Upper Siwalik subgroup and are associated with the upper Tatrot faunal zone and the lower Pinjor faunal zone. Two laterally persistent bentonitized tuffs occur in the Jhelum area (2.40 ± 2.0 Ma and 2.58 ± 0.06 Ma)[2]. In the Jammu area, a fission track age of 2.8 ± 0.56 Ma for the upper tuff of the Nagrota Formation from Uttarbeni has been mentioned[3]. Another bentonitic tuff from the Nagrota section of the Jammu Hills has given a zircon FT age of 2.31 ± 0.54 Ma (ref. 3). These tuffs have been equated[3] with the tuff couplet occurring along the Gauss–Matuyama boundary in the Siwalik Belt of Pakistan. A FT age of 1.6 ± 0.2 Ma from a bentonite bed in the Nagrota Formation of Uttarbeni area is also reported[4]. FT analysis of zircon grains from a bentonitic tuff bed of Uttarbeni in the present study has given an age of 1.59 ± 0.32 Ma (Table 1). This is at variance with the age reported by Tripathi[4] but is lower than those of Ranga Rao *et al.*[3].

The tuffaceous mudstones of the Pinjor Formation in the Ghaggar section may, on the basis of FT dating of zircon separates, be related to the Gauss–Matuyama boundary. Further understanding of the chronostratigraphy of the Upper Siwalik subgroup will require integration of the fission track data with magnetic polarity stratigraphic and rodent biochronologic data.

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A potential immunosuppressive role for vasoactive intestinal peptide (VIP) in leishmaniasis: Evidence from the use of a *selective VIP antagonist*

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The immunological relevance of vasoactive intestinal peptide (VIP) in the pathology of leishmaniasis has been tested. *Leishmania*-primed mouse-derived splenic cells were examined and found to exhibit suppressed and upregulated mitogenic responses in the absence and the presence of VIP respectively. Furthermore, plasma derived from *Leishmania*-primed hamsters induced a suppressive effect on the mitogenic response of normal hamster splenic cells; this was significantly blocked by a selective VIP antagonist. The results showed the functional presence of VIP at the level of immunological disorder within a *Leishmania*-primed milieu.

THE encapsulation of the *Leishmania* parasites within the macrophage phagolysosomal vacuole of a susceptible mammalian host, harbingers a spectrum of immunological abnormalities[1-4] which favour the evolution of a defective cell-mediated immune response towards increased parasitism and disease. Parasite innate ability to effect these changes is largely ascribed to its surface antigenic molecules (membrane bond and excreted forms) such as the lipophosphoglycan (LPG), glycoprotein 63 (gp63) and acid phosphatase (AP) (see refs. 5–7). In addition, a host of serum-associated factors such as the prostaglandins, immunoglobulins, triglycerides and protein molecules[8,9], generally released in increasing quantity during infection, have also been described as important contributory molecules in the dysregulation of host immune cell functions in leishmaniasis. Indeed, speculation is that immunosuppressive factors in serum/plasma sustain the process of immunosuppression[10,11]. The functional significance accorded these factors in the overall process of immunosuppression, prompted consideration for the role of a potent immunosuppressive endogenous peptide molecule, the vasoactive intestinal peptide[12] (VIP), in the process.

The VIP is thought to influence the pathophysiology of certain tumour-related diseases[13,14] and parasitic infections[15,16]. In recent times, it has gained prominence as a molecule involved in the exacerbation of disease processes through an immunological axis[14,17], given its ability to inhibit several immunological functions such as monocyte oxidative activity[18], lymphocyte mitogenic response[19,20] and the release of lymphokines[21], and natural killer (NK) cell activity[22]. Indeed, VIP is viewed as potent in the dysregulation of host immune cell response in the human immunodeficiency virus (HIV) infection[17,23] on the basis of the presence of VIP-like substances in serum of HIV-positive patients, coupled with the resemblance of VIP and HIV-induced immunological features. Similarly, infections by *Leishmania* parasites are also associated with immunological features that parallel those induced by VIP[1-4,18-22]. In this respect, VIP may be relevant in the overall processes that tilt host immune response towards anergy in leishmaniasis.

This paper reports that splenic cells derived from immunosuppressed *Leishmania*-primed BALB/c mice exhibit certain degree of sensitivity to VIP *in vitro* and that VIP-like substances are functionally detectable in plasma of immunosuppressed *Leishmania*-primed hamsters. The results suggest that VIP is functionally present at the level of immunological disorder in leishmaniasis.

VIP (complete 28 amino acid porcine sequence), VIP_{10-28} (carboxyl partial sequence of VIP—as VIP antagonist at the receptor level[24,25]) and Aprotinin were obtained from Sigma. Radioactive labelled thymidine (^{3}H-TdR, specific activity 6.5 curie/mM) was obtained from the Bhabha Atomic Research Centre, Bombay, India. Strains of *Leishmania donovani* and *L. tropica* were obtained from the Department of Immunopathology, Post Graduate Institute of Medical Research, Chandigarh.

Experiment one: *Leishmania*-primed mouse-derived splenic cell response to mitogen in the absence or presence of VIP.

Seven-day-old cultures of *L. donovani* and *L. tropica* promastigotes were washed and suspended on 0.15 M PBS, pH 7.2, and thereafter killed by repeated freezing and thawing (5 times) for particulate antigens[26]. Sets of 2 BALB/c mice (10 g) were challenged, at various times, with 0.1 ml of promastigote suspension, equivalent to 10^8 *L. donovani* and 10^7 *L. tropica* particulate antigens, with complete Freund's adjuvant (CFA) by subcutaneous inoculation at a shaved area at the base of the tail. Seven days post-initial-challenge, mice were rechallenged with 0.1 ml of 10^6 particulate antigen suspension admixed with incomplete Freund's adjuvant (IFA). Control mice (3) were inoculated with CFA and IFA